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9904804.3 **2 March 1999 (02.03.1999)** **GB**
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(54) Title: **IDENTIFICATION OF BACTERIA BY AMPLIFICATION AND PROBING**

(57) Abstract: A method for identifying bacteria in a sample is described which comprises amplifying a portion of the 23S rDNA present in the sample using, as one primer, a degenerate primer set comprising one or more DNA molecules consisting essentially of DNA having the sequence(s) 5'GCGATTTCYGAAYGGGGRAACCC, the other primer consisting of DNA having the sequence 5'TTCGCCTTTCCCTCACGGTACT and testing the resulting amplicon by hybridisation to one or more oligonucleotide probes designed to identify one or more bacteria likely to be present in the sample. The method allows for the identification of at least 8 and considerably more bacterial species in a single test, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus* spp., *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp, *Pneumococci*, and coagulase negative *Staphylococci*. One or more novel oligonucleotides for use in this test are immobilised on a solid carrier and incorporated in a diagnostic test kit for use in hospitals and other environments.

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INTERNATIONAL SEARCH REPORT

International Application No

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A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, STRAND, WPI Data, PAJ, MEDLINE, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 00298 A (INNOGENETICS NV ;JANNES GEERT (BE); ROSSAU RUDI (BE); HEUVERSWYN H) 4 January 1996 (1996-01-04)	1-5, 10-12, 14,15, 25,27
Y	* see especially page 64, line 20 as well as SEQ ID NO:156 * the whole document	17-19, 21,22, 26,27
X	EP 0 395 292 A (BARRY THOMAS GERARD ;GANNON BERNARD FRANCIS XAVIER (IE); IRELAND B) 31 October 1990 (1990-10-31) * see especially claims 1-7 * the whole document	1-5,10, 11

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/00740

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	LEW A E AND DESMARCHELIER P M: "Detection of Pseudomonas pseudomallei by PCR and hybridization" JOURNAL OF CLINICAL MICROBIOLOGY, vol. 32, no. 5, 1994, pages 1326-1332, XP000921285 the whole document ---	1,10,11
X	WO 96 24686 A (BIO MERIEUX ;MABILAT CLAUDE (FR); SALLEN BRUNEHILD (FR)) 15 August 1996 (1996-08-15) abstract; example 1 ---	14
X	WO 88 03957 A (GEN PROBE INC) 2 June 1988 (1988-06-02) ---	14,15, 17,19, 25,26
Y	* see especially claims 222 and 195 * the whole document ---	1-6,8, 10-12
X	WO 90 14444 A (GENE TRAK SYSTEMS) 29 November 1990 (1990-11-29) ---	17,19
Y	* see especially page 9, line 21 * the whole document ---	1,3,5,6, 8,12,21, 22
Y	US 5 521 300 A (SHAH JYOTSNA S ET AL) 28 May 1996 (1996-05-28) ---	1-4,6-8, 10-12, 17-19, 21,22, 26,27
	* see especially COLUMN 83, SEQ ID NO:47 * the whole document ---	
Y	LUDWIG W ET AL.: "PCR-based preparation of 23S rRNA-targeted group-specific polynucleotide probes" APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 60, no. 9, 1994, pages 3236-3244, XP000921283 the whole document ---	1-8, 10-12
A	US 5 582 978 A (SHAH JYOTSNA) 10 December 1996 (1996-12-10) the whole document ---	
P,X	US 6 001 564 A (BERGERON MICHEL G ET AL) 14 December 1999 (1999-12-14) * see especially column 9, paragraph 1 * the whole document -----	1-20, 25-27

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 00/00740

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

As a result of the prior review under R. 40.2(e) PCT,
part of the additional fees are to be refunded.

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
1-8, 10-19, 21-27
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☒ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 13-16,25 (complete); 1-6,8,10-12,17,19,21-24,26, 27 (partial)

INVENTION 1:

A primer set, suitable for amplification of bacterial 23S rRNA, comprising SEQ ID Nos 1 or 2, a labelled DNA sequence comprising such a sequence, an oligonucleotide probe according to SEQ ID Nos:3-4, suitable for detecting Proteus species, a solid support material carrying such probe(s), a diagnostic kit comprising such primers and/or probe(s), as well as a method of identifying bacteria using such primers and probe(s).

2. Claims: 1-8,10-12,17-19,21-24,26,27 (partial)

INVENTION 2:

An oligonucleotide probe according to SEQ ID Nos:5,8,10,37,48, suitable for detecting E.coli species, a solid support material carrying such probes, a diagnostic kit comprising such probe(s), as well as an amplification method of identifying bacteria using such probe(s).

3. Claims: 1-4,6,8,10-12,17,19,21-24,26,27 (partial)

INVENTION 3:

An oligonucleotide probe according to SEQ ID Nos:6,7, suitable for detecting Klebsiella species, a solid support material carrying such probes, a diagnostic kit comprising such probe(s), as well as an amplification method of identifying bacteria using such probe(s).

4. Claims: 1-8,10-12,17-19,21-24,26,27 (partial)

INVENTION 4:

An oligonucleotide probe according to SEQ ID Nos:9,38,49, suitable for detecting Enterobacter species, a solid support material carrying such probes, a diagnostic kit comprising such probe(s), as well as an amplification method of identifying bacteria using such probe(s).

5. Claims: 1,2,4,6,8,10-12,17,19,21-24,26,27 (partial)

INVENTION 5:

An oligonucleotide probe according to SEQ ID NO:11, suitable for detecting Salmonella species, a solid support material carrying such probe, a diagnostic kit comprising such probe, as well as an amplification method of identifying bacteria

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

using such probe.

6. Claims: 1,2,4,6-8,10-12,17-19,21-24,26,27 (partial)

INVENTION 6:

An oligonucleotide probe according to SEQ ID Nos:12,15,18,30-35, suitable for detecting Streptococcus species, a solid support material carrying such probes, a diagnostic kit comprising such probe(s), as well as an amplification method of identifying bacteria using such probe(s).

7. Claims: 1-6,8,10-12,17,19,21-24,26,27 (partial)

INVENTION 7:

An oligonucleotide probe according to SEQ ID NO:13, suitable for detecting Pseudomonas species, a solid support material carrying such probe, a diagnostic kit comprising such probe, as well as an amplification method of identifying bacteria using such probe.

8. Claims: 1,2,4,7,8,10-12,18,19,21-24,26,27 (partial)

INVENTION 8:

An oligonucleotide probe according to SEQ ID NO:14, suitable for detecting Haemophilus species, a solid support material carrying such probe, a diagnostic kit comprising such probe, as well as an amplification method of identifying bacteria using such probe.

9. Claims: 1-6,8,10-12,17,19,21-24,26,27 (partial)

INVENTION 9:

An oligonucleotide probe according to SEQ ID Nos:16,19, suitable for detecting Enterococcus species, a solid support material carrying such probes, a diagnostic kit comprising such probe(s), as well as an amplification method of identifying bacteria using such probe(s).

10. Claims: 1,2,4,6,8,10-12,17,19,21-24,26,27 (partial)

INVENTION 10:

An oligonucleotide probe according to SEQ ID NO:17, suitable for detecting Aeromonas species, a solid support material carrying such probe, a diagnostic kit comprising such probe, as well as an amplification method of identifying bacteria using such probe.

11. Claims: 1-6,8,10-12,17,19,21-24,26,27 (partial)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

INVENTION 11:

An oligonucleotide probe according to SEQ ID Nos:20-26, suitable for detecting Staphylococcus species, a solid support material carrying such probes, a diagnostic kit comprising such probe(s), as well as an amplification method of identifying bacteria using such probe(s).

12. Claims: 1,2,4-6,8,10-12,17,19,21-24,26,27 (partial)

INVENTION 12:

An oligonucleotide probe according to SEQ ID NO:27, suitable for detecting Burkholderia species, a solid support material carrying such probe, a diagnostic kit comprising such probe, as well as an amplification method of identifying bacteria using such probe.

13. Claims: 1,2,4,6,8,10-12,17,19,21-24,26,27 (partial)

INVENTION 13:

An oligonucleotide probe according to SEQ ID NO:28, suitable for detecting Stenotrophomonas species, a solid support material carrying such probe, a diagnostic kit comprising such probe, as well as an amplification method of identifying bacteria using such probe.

14. Claims: 1,2,4,5,7,8,10-12,18,19,21-24,26,27 (partial)

INVENTION 14:

An oligonucleotide probe according to SEQ ID NO:29, suitable for detecting Listeria species, a solid support material carrying such probe, a diagnostic kit comprising such probe, as well as an amplification method of identifying bacteria using such probe.

15. Claims: 1,2,4,7,8,10-12,18,19,21-24,26,27 (partial)

INVENTION 15:

An oligonucleotide probe according to SEQ ID NO:36, suitable for detecting Acinetobacter species, a solid support material carrying such probe, a diagnostic kit comprising such probe, as well as an amplification method of identifying bacteria using such probe.

16. Claims: 1,2,4,6-8,10-12,17-19,21-24,26,27 (partial)

INVENTION 16:

An oligonucleotide probe according to SEQ ID Nos:38,39, suitable for detecting CNS species, a solid support material carrying such probes, a diagnostic kit comprising such

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

probe(s), as well as an amplification method of identifying bacteria using such probe(s).

17. Claims: 12,4,6,8,10-12,17,19,21-24,26,27 (partial)

INVENTION 17:

An oligonucleotide probe according to SEQ ID NO:41, suitable for detecting Plesiomonas species, a solid support material carrying such probe, a diagnostic kit comprising such probe, as well as an amplification method of identifying bacteria using such probe.

18. Claims: 1,2,4,7-12,18-24,26,27 (partial)

INVENTION 18:

An oligonucleotide probe according to SEQ ID Nos:42,43,60,61, suitable for detecting Neisseria species, a solid support material carrying such probes, a diagnostic kit comprising such probe(s), as well as an amplification method of identifying bacteria using such probe(s).

19. Claims: 1,2,4,6,8,10-12,17,19,21-24,26,27 (partial)

INVENTION 19:

An oligonucleotide probe according to SEQ ID Nos:44,45, suitable for detecting Campylobacter species, a solid support material carrying such probes, a diagnostic kit comprising such probe(s), as well as an amplification method of identifying bacteria using such probe(s).

20. Claims: 1,2,4,6,8,10-12,17,19,21-24,26,27 (partial)

INVENTION 20:

An oligonucleotide probe according to SEQ ID NO:46, suitable for detecting Helicobacter species, a solid support material carrying such probe, a diagnostic kit comprising such probe, as well as an amplification method of identifying bacteria using such probe.

21. Claims: 1,2,4,6,8,10-12,17,19,21-24,26,27 (partial)

INVENTION 21:

An oligonucleotide probe according to SEQ ID NO:47, suitable for detecting Ralstonia species, a solid support material carrying such probe, a diagnostic kit comprising such probe, as well as an amplification method of identifying bacteria using such probe.

22. Claims: 1,2,4,6-12,17-24,26,27 (partial)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

INVENTION 22:

An oligonucleotide probe according to SEQ ID Nos:50-52,62,63, suitable for detecting Chlamydia species, a solid support material carrying such probes, a diagnostic kit comprising such probe(s), as well as an amplification method of identifying bacteria using such probe(s).

23. Claims: 1,2,4,6,8,10-12,17,19,21-24,26,27 (partial)

INVENTION 23:

An oligonucleotide probe according to SEQ ID NO:53, suitable for detecting Coxiella species, a solid support material carrying such probe, a diagnostic kit comprising such probe, as well as an amplification method of identifying bacteria using such probe.

24. Claims: 1,2,4,6,8,10-12,17,19,21-24,26,27 (partial)

INVENTION 24:

An oligonucleotide probe according to SEQ ID Nos:54,55, suitable for detecting Rhodococcus species, a solid support material carrying such probes, a diagnostic kit comprising such probe(s), as well as an amplification method of identifying bacteria using such probe(s).

25. Claims: 1,2,4,6,8,10-12,17,19,21-24,26,27 (partial)

INVENTION 25:

An oligonucleotide probe according to SEQ ID Nos:56-58, suitable for detecting Mycobacterium species, a solid support material carrying such probes, a diagnostic kit comprising such probe(s), as well as an amplification method of identifying bacteria using such probe(s).

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

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